

A NEW TRI-HYBRID LYCOPOD,
DIPHASIASTRUM DIGITATUM × *SABINIFOLIUM*

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ABSTRACT

A putative tri-specific hybrid *Diphasiastrum* (Lycopodiaceae) is described from a collection in northern Vermont and is figured in a line drawing. The hybrid is morphologically intermediate between its probable parents, *D. digitatum* and *D. × sabinifolium*. This is the first flat-branched hybrid lycopod believed to involve three parental genomes. In light of its limited occurrence (a single clone), it is not given an epithet.

Key Words: *Diphasiastrum* (*Lycopodium*), hybrid, genome analysis, species concept, Vermont

INTRODUCTION

Five species, and at least five hybrids, of the “flat-branched” lycopods (*Diphasiastrum* Holub) are known in northeastern North America (Table 1). The taxonomy of this group has to date relied almost entirely on morphology. Hybrids generally display characters intermediate between their parents, and are usually distinguishable in the field and herbarium on morphological grounds (Wilce, 1965). Cytological work has indicated that all North American species and hybrids share a chromosome number $2n = 46$ (F. S. Wagner, 1992), with apparent normal pairing behavior and spore formation in the hybrids (the plant described here produces well-formed spores). Additionally, the difficulty of germinating spores *in vitro* and growing the resulting plants has so far precluded artificial crossing experiments in *Diphasiastrum*, and the genus as a whole has not been analyzed on the basis of isozymes or DNA. On morphological grounds, Wilce (1965) determined *D. × sabinifolium*, which had long been considered a species, to be the hybrid *D. sitchense* × *tristachyum*, a treatment which has gained acceptance (Beitel, 1979; Cody and Britton, 1989; Wagner and Beitel, 1993). Except for *D. × issleri*, which is known in our area from a single collection, and *D. complanatum* × *digitatum*, which may be quite rare, the hybrids are sometimes locally common (Cody and Britton, 1989; W. H. Wagner, pers. comm.), and *D. × sabinifolium* sufficiently so to be regarded as a nothospecies. The hybrids appear to be fertile (F. S. Wagner, 1992), yet no hybrid progeny or backcrosses with parent species

Table 1. Species and hybrids of *Diphiastrum* in northeastern North America (all $2n = 46$).

Species
<i>D. digitatum</i> (Dill.) Holub
<i>D. complanatum</i> (L.) Holub
<i>D. tristachyum</i> (Pursh) Holub
<i>D. sitchense</i> (Rupr.) Holub
<i>D. alpinum</i> (L.) Holub
Hybrids
<i>D. × sabinifolium</i> (Willd.) Holub (<i>sitchense</i> × <i>tristachyum</i>)
<i>D. × habereri</i> (House) Holub (<i>digitatum</i> × <i>tristachyum</i>)
<i>D. complanatum</i> × <i>digitatum</i>
<i>D. × zeilleri</i> (Rouy) Holub (<i>complanatum</i> × <i>tristachyum</i>)
<i>D. × issleri</i> (Rouy) Holub (<i>alpinum</i> × <i>tristachyum</i>)

have been described. This paper discusses a new hybrid, *D. digitatum* × *sabinifolium*, at present known from a single station in northern Vermont. It is the first hybrid known to have a hybrid parent.

HYBRID DESCRIPTION AND ANALYSIS

While collecting a series of pteridophytes in Caledonia County, Vermont, I discovered, in a montane (ca. 540 m) east-facing pasture, an unusual clone of *Diphasiastrum* which was not readily referable to any described taxon. It was growing with *D. digitatum* and *D. tristachyum* but was clearly neither. Collections were made in August and November, 1993, from the same clone.

This plant has the general aspect of *D. × sabinifolium* with a congested, tufted habit rather than a pseudo-monopodial habit such as characterizes *D. digitatum* and *D. tristachyum* (Figure 1). This is due to the branching of upright stems near their bases and to the length of the lower branches which reach beyond the bases of upper branches. The plant is also similar to *D. × sabinifolium* in its short, usually unbranched peduncle with one or two sporophylls on the peduncle below the strobilus. A hybrid origin, however, was suspected because the lateral branchlets are strongly dorsiventrally flattened, the lateral leaves are appressed rather than spreading, and the lower (ventral) leaves are reduced, all characteristics that do not occur in *D. × sabinifolium*. Fur-

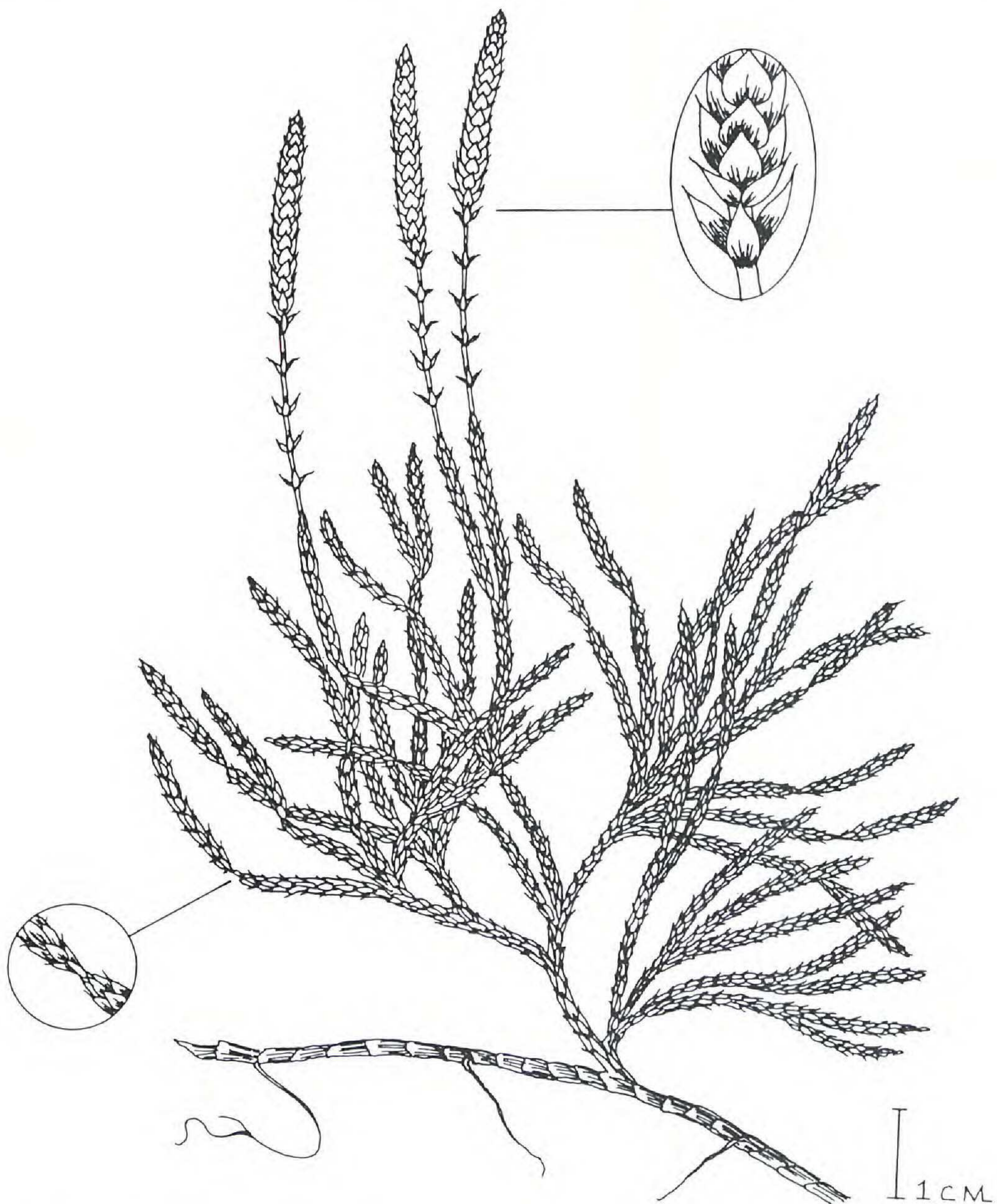


Figure 1. *Diphasiastrum digitatum* \times *sabinifolium*. The influence of *D.* \times *sabinifolium* is indicated by the low branching, the indeterminate growth of branches as shown by annual constrictions, and the unbranched peduncle with sporophylls scattered at the base of the strobilus (see details). The broad, flattened branches, reduced lower leaves and broad sporophylls are evidence of *D. digitatum* parentage.

thermore, the plant overall is larger and stouter than typical *D.* \times *sabinifolium*.

A morphological analysis was undertaken to determine the identity of this puzzling plant. With such tests of hybridity as garden experiments, isozyme or DNA analysis lacking at present,

Table 2. Hybrid index comparing ten qualitative characters of the putative hybrid *D. digitatum* × *sabinifolium* and its parents. (2 = character as in *D. × sabinifolium*; 1 = intermediate; 0 = character as in *D. digitatum*).

Character (from Wilce, 1965)	sab	dig × sab	dig
1. Habit congested due to branching low on upright stem, vs. pseudomonopodial	2	1	0
2. Branches indeterminate for 2 or more years, as shown by annual constrictions	2	2	0
3. Branches slightly glaucous, vs. bright green	2	1	0
4. Branches not or only slightly flattened dorsiventrally	2	0	0
5. Lower leaves not reduced, vs. reduced	2	1	0
6. Lateral leaves spreading vs. appressed	2	0	0
7. Lateral leaves with narrow base, vs. broad base	2	0	0
8. Fertile branch round with equal leaves (vs. flattened with reduced lower leaves)	2	2	0
9. Peduncle usually unbranched (vs. normally branched)	2	2	0
10. Sporophyll narrow (vs. broad)	2	0	0
Total	20	9	0

therefore, morphological comparisons give the strongest evidence of this plant's hybrid status.

A series of hybrid indices (modified from Wilce, *op. cit.*, pp. 112–114) were prepared to compare this plant with potential parents. From these, it was determined that *D. digitatum* and *D. × sabinifolium* are the most likely parents. Table 2 is the index prepared on the basis of ten qualitative characters by which these probable parents differ. Of the ten, four character states (irregular, tufted habit; indeterminate branchlet growth; rounded fertile branch with isomorphic leaves; and unbranched peduncle) are closest to *D. × sabinifolium*. Six characters (green coloration; dorsiventrally flattened and appressed lateral leaves; broad bases of lateral leaves, reduced lower leaves, broad sporophylls) are closer to *D. digitatum*. The putative hybrid has an index score of 9, intermediate between the scores of *D. digitatum* (0) and *D. × sabinifolium* (20).

Quantitative characters give a somewhat less clear picture (Table 3), since the hybrid is not medial in every character, but approaches one parent or the other, or exceeds the parents in

Table 3. Comparison of mean quantitative morphological characters, each based on 30 measurements of dried plants (*D. digitatum*, Gilman 93241, Wheelock, VT; *D. digitatum* × *sabinifolium*, Gilman 93246, Walden, VT and Gilman 92069, Marathon, Ont.). All measurements in mm. Standard deviations in parentheses.

Character	sab	dig × sab	dig
1. Branch width	1.42 (0.25)	2.01 (0.68)	2.52 (0.57)
2. Lower leaf width (base of free portion)	0.71 (0.09)	0.84 (0.12)	0.53 (0.12)
3. Lower leaf length (free portion)	2.28 (0.28)	1.32 (0.15)	1.05 (0.14)
4. Ratio of lower leaf width/length	0.311	0.636	0.505
5. Peduncle length	24.6 (7.2)	28.8 (6.24)	48.5 (7.6)
6. Strobilus length	21.6 (4.24)	30.6 (4.78)	22.0 (1.40)
7. Sporophyll width	2.28 (0.28)	2.02 (0.14)	1.88 (0.17)
8. Sporophyll length	2.08 (0.08)	1.98 (0.10)	1.84 (0.09)
9. Ratio of sporophyll width/length	1.09	1.02	1.02

a few cases. Branchlet width at 2.01 mm is intermediate between the parents. The free portions of the lower leaves are intermediate in length, but are somewhat wider than either parent. The peduncle length is intermediate, but closer to *D. × sabinifolium*. Strobilus length is greater than either parent, indeed greater than average for any native species (Wilce, 1965); this may be an anomaly of the individual plant or may be the result of hybrid vigor. The sporophyll is intermediate in width and length, but approaches *D. digitatum* in ratio of width to length (the “complanatum type” of Wilce).

DISCUSSION

Accepting the hypothesis that *D. × sabinifolium* is of hybrid origin, then this hybrid has three ancestral genomes, *D. digitatum*, *D. sitchense*, and *D. tristachyum*, presumably present in the proportion 2:1:1.

Deviations from some expected intermediate character states may be inherited from individually variable parents. *D. × sabinifolium* is itself quite variable (Cody and Britton, 1989) and variations in certain characters may have been passed on to the hybrid. Alternatively, variations may be due to unequal contributions of the ancestral genomes, *D. digitatum*, *D. sitchense* and *D. tristachyum*. In particular, *D. sitchense* seems to be strongly

represented in the short unbranched peduncle (as opposed to the long and regularly forked peduncles of *D. digitatum* and *D. tristachyum*), and in the low-branched, tufted habit (as opposed to the pseudomonopodial habit of *D. digitatum* and *D. tristachyum*).

Another potential hybrid combination, between *D. sitchense* and *D. × habereri* would have the same genomes in different proportion (1:2:1). On analogy with *D. × sabinifolium*, such a hybrid should display *D. sitchense* characters even more strongly, i.e., it would be expected to have non-flattened branches, spreading lateral leaves, and unreduced lower leaves. Furthermore, this alternative is unlikely because neither parent is known in the immediate vicinity, whereas *D. digitatum* is abundant in the area and *D. × sabinifolium* is known to occur nearby (D.S. Barrington, pers. comm.).

As noted above, species and hybrids in *Diphasiastrum* are morphologically distinct, and the hybrids are apparently fertile. The barriers that prevent the hybrids from back-crossing and rehybridizing to the point of swamping the parent species, which maintain their distinctions over broad ranges, have not been identified. The discovery of the hybrid discussed here is significant because it suggests at least the possibility of introgression and the formation of hybrid swarms. On the other hand, both its distinctiveness and its rarity confirm the current concepts of species within the group.

Although its spores are well-formed and may be viable, this hybrid is as yet known only from one site. There is no direct evidence that it is reproductively competent beyond surviving and slowly spreading as a single clone. For this reason I have chosen not to provide an epithet.

MATERIAL EXAMINED

Vermont: **Caledonia County**: Wheelock, highest pasture on E side of Ide Mountain, elevation ca. 540 m, *A. V. Gilman* 93240, 21 August and 27 November 1993 (VT, NEBC, MICH).

ACKNOWLEDGMENTS

The author thanks Warren H. Wagner Jr. and David S. Barrington for discussions concerning the nature of this plant, and Martha S. Brown for preparation of Figure 1; also two anonymous reviewers for comments.

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